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APPLICATION NUMBER: *60/544,356*
FILING DATE: *February 17, 2004*

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
MORDECHAI ASSAF	DEUTSCH DEUTSCH	MOSHAV OLESH, ISRAEL TZAFARIA, ISRAEL

Additional Inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

MICRO-FLUIDICS INTEGRATED POLYMERIC INDIVIDUAL CELL CHIP DESIGN, MANUFACTURING AND SAMPLES OF APPLICATIONS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ Customer Number:

OR

☑ Firm or Individual Name	SCHOTTENSTEIN CELLOME RESEARCH CENTER				
Address	BAR ILAN UNIVERSITY				
Address					
City	RAMAT GAN	State	ISRAEL	Zip	52900
Country	ISRAEL	Telephone	97235344675	Fax	97235342019

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 6
☑ Drawing(s) *Number of Sheets* 3
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number _____
☐ Other (specify) _____

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.
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Respectfully submitted,

SIGNATURE _____ Date 2/17/04

TYPED or PRINTED NAME MORDECHAI DEUTSCH REGISTRATION NO. _____ *(if appropriate)*

TELEPHONE 97235344675 Docket Number: 34

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

[X] Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80

Complete if Known	
Application Number	
Filing Date	2/17/2004
First Named Inventor	MORDECHAI DEUTSCH
Examiner Name	
Art Unit	
Attorney Docket No.	34

METHOD OF PAYMENT (check all that apply)

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80

SUBTOTAL (1) ($) 80

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below	Fee Paid
Total Claims	-20** =		X	
Independent Claims	-3** =		X	
Multiple Dependent				

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

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SUBTOTAL (3) ($)

SUBMITTED BY

(Complete if applicable)

Name (Print/Type)	ROBERT VASE	Registration No. (Attorney/Agent)	Telephone 972353446 75
Signature			Date 2/17/04

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PROVISIONAL PATENT APPLICATION

Title: Micro-fluidics Integrated Polymeric individual cell chip design, manufacturing and samples of applications.

Background

The current glass-based INTERACTIVE TRANSPARENT INDIVIDUAL CELLS BIOCHIP PROCESSOR (ITICBP) (individual cell chip) (WO 03/035824) posses some unique unmatched advantages:

1. The ability to preserve the location of certain viable cells in certain wells during long periods of time.
2. The ability to flush the cells with various dyes and reagents, yet saving the cells location.
3. The ability to watch, monitor and perform interactive tests on individual cells at any time.

However, the current production of the current glass-made ITICBP is relatively complex, costly and time consuming. The process consists of delicate and precise steps, starting with individually etching and cutting every piece of glass and gaskets, followed by a slow manual assembly process of the glass pieces, gaskets, tubing and adhesives altogether. Moreover, it is not simple to make changes in the basic design of the micro-fluidics system, made of external metal needles attached to the ITICBP.

A brief description of the invention:

The present invention (hereinafter: Polymeric-ITICBP or P-ITICBP) refers to a novel micro-fluidics integrated ITICBP design and manufacturing, based upon easily replicable components and simple assembly processes. The proposed invention paves the way for easy reproduction of ITICBP by simplifying the manufacturing process of the cell-wells array and featuring inherent micro-fluidics routes. Consequently, the proposed invention represents a robust ITICBP design, that is less sensitive to impacts and jolts and thus more suitable for shipping.

The present invention takes advantage of the casting method that is widely known as soft photolithography. Soft photolithography, i.e. casting a resilient polymer against preset solid mold, is a known and widely used method in lab-on-chip applications as

well as in micro-fluidics applicati ns. Poly-DiMethylSiloxane (PDMS) is one of the most common materials for soft photolithography, since PDMS has the capability of exact replication of delicate microstructures as well as the convenience of simple, removal from the mold without leaving unwanted residue thereuopon.

PDMS is characterized by good optical transparency, low fluorescence, thermal and environmental stability and chemical inertness with respect to most laboratory reagents. Furthermore, PDMS is non-toxic using accepted cyto-toxicity assays. For example, formerly frozen human prostate cancer cells (PC3 Prostate cancer cells, from DSMZ GmbH, Germany), were cultured in RPMI medium with 10% Fetal calf Serum (FCS). Twice a week the cells were re-located to a new dish of identical type (control or silicone), by using trypsin-EDTA to detach the cells from the bottom. Trypsin-EDTA was applied for 1 minute in 37*c incubator. After trypsin - EDTA treatment, medium with serum was added to stop trypsin-EDTA activity, and new medium with serum is added. Two types of PDMS yield the following data:

Silicone #1 - RTV615 from GE Silicones, **Silicone #2** - Sylgard 184 from Dow-Corning. **Control** – standard petri dish (Nunclon from Nunc, denmark):

Incubation Time	24h	48h	72h	96h
Control	Adherence	Growth starts	Growth	Growth
Silicone #1	No adherence	Adherence	Growth starts	Growth
Silicone #2	No adherence	Adherence	Growth starts	Growth

After re-locating the cells on fresh, identical dishes:

Time from re-location	24h	48h	72h	96h
Total Elapsed time	120h	144h	168h	192h
Control	Adherence	Growth starts	Growth	Growth
Silicone #1	No adherence	No adherence	Adherence	Growth starts
Silicone #2	No adherence	No adherence	Adherence	Growth starts

It is important to note that while cell adherence and growth were postponed, no cell death was observed during the experiment.

Description of preferred embodiments

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting

The invention includes a manufacturing process. The first step in the manufacturing process is making the mold with embossed micro-patterns (i.e. excess of material on the mold surface) of the cell wells, interconnecting plumbing routes and reservoirs. The mold may be manufactured using known methods of micro-modeling, e.g. photolithography, micro-etching, micro-plating, etc. The mold may be fashioned from any solid material that is known in the art for molding such as nickel.

Figure 1 depicts an example of a simple Nickel mold of cell wells array and demonstrates the microstructure of the embossed Nickel domes.

Figure 2 demonstrates a more complex mold design, harboring microfluidics vessels and reservoirs.

Preferably, the mold is surface-treated to reduce adherence.

In a subsequent step, the liquid PDMS is cast upon the mold. After de-aeration, vulcanization (=polymerization) may take place in room temperature or be accelerated by heating up to 150°C. As vulcanization is accomplished, the cast may be fully peeled off the mold, resulting in a PDMS slab engraved with cell wells, plumbing routes and reservoirs.

Figures 3 (a) and 3(c) depict the high quality of the replication process that forms the P-ITICBP from mold, with regards to the standard glass ITICBP (Figure 3 (b)).

The P-ITICBP resulting from the manufacturing process may be used with the open side of the wells and plumbing routes face up. The plumbing routes are actually "U" or "V" shaped thus it is advised that their open end should be sealed in order to create a closed fluid system (Figure 3(d)).

The PDMS casting may be placed on a microscope slide, and covered with a cover slip. Adhesion of the cover slip to the PDMS seals the internal cavity of the P-ITICBP (wells and plumbing).

Optionally, but preferably, every plumbing route is terminated with a larger diameter reservoir, in order to enable easy access to the reservoir by using a small diameter hollow metal needle or plastic tubing to form an input/output port to an external fluid management system.

The P-ITICBP may be used in a complex system for controlled cells flow to the chip together with controlled staining of the cells. For example, a laser tweezers system (i.e. highly focused laser beam capable of trapping micron-sized particles and living cells in a liquid medium) may be incorporated in order to manipulate and sort different types of cells.

Figure 4 depicts the use of laser tweezers to selectively load, re-locate and manipulate living cells in the cell wells of the P-ITICBP.

The P-ITICBP can take a significant role in systems where several dyes or reagents should flush the cells. For example, a computer-controlled system can pump cells into the P-ITICBP and monitor the amount and timing of various fluids, as required for a specific test protocol. Along this time, the cells are placed in their wells, keeping their specific location and may be observed through the microscope (Figure 5).

Figure 6 depicts a system that can activate laser tweezers to sort cells according to preset characteristics. The laser tweezers trap individual cells and separate them into different reservoirs within the P-ITICBP. By pumping fluids into or from the reservoirs, the separated cells can then be collected to external vessels.

A combination of the systems described in figures 4 and 5 is an especially preferred embodiment of the invention.

According to some preferred embodiments, two or more P-ITICBP chips and/or plumbing routes are stacked vertically.

According to additional preferred embodiments a various number of input and output ports, having identical or various diameters are employed.

According to additional preferred embodiments micro-valves and/or pumps are used in conjunction with the P-ITICBP.

According to additional preferred embodiments various combinations and designs of well sizes, interconnecting plumbing routes and reservoirs are employed.

According to additional preferred embodiments adhesives and/or plasma treatment are employed to adhere PDMS to PDMS, glass or other materials.

According to additional preferred embodiments PU (Polyurethane) is employed to construct the P-ITICBP.

According to additional preferred embodiments staining of certain marking points (fiducial points) on the P-ITICBP aids in calibration of orientation of the ITICBP during observation.

According to additional preferred embodiments staining certain marking points (fiducial points) on the P-ITICBP mold to be transferred to the PDMS during casting (to calibrate the orientation of the P-ITICBP during observation).

According to additional preferred embodiments the staining is accomplished with visible dye or fluorescent dye.

According to additional preferred embodiments the P-ITICBP is used in conjunction with manual or computer-controlled fluid management system.

According to additional preferred embodiments the P-ITICBP is used in conjunction with a laser tweezers system.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad

scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

CLAIM: A Micro-fluidics Integrated Polymeric Individual Cell Chip system essentially as described herein above or depicted in the drawings.

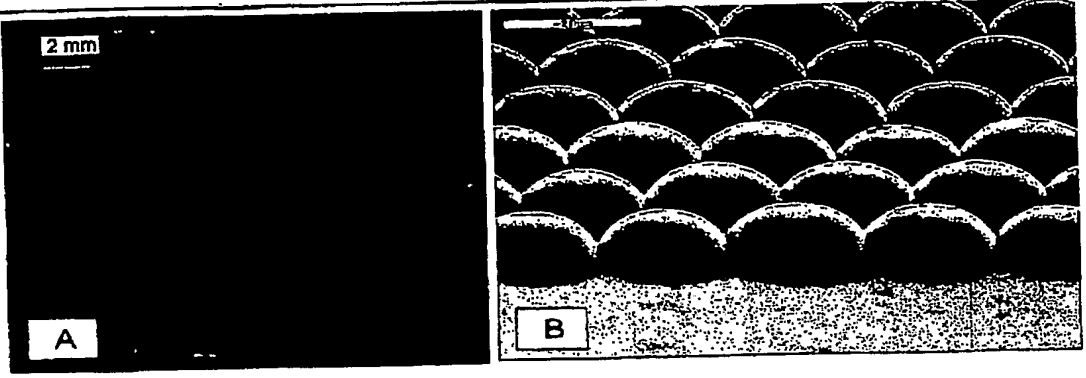

Figure 1:
(1st) A micro-plated Nickel mold of four separated 100*100 cell-wells arrays.
(2nd) A SEM micrograph of the domes on the Nickel plate, that will produce the cell – wells in the PDMS replica.

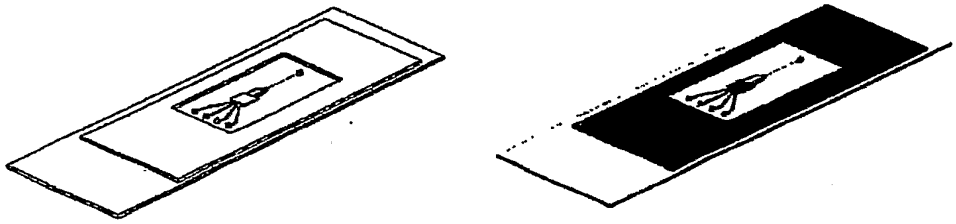

Figure 2: A sample of an embossed ITICBP mold design, featuring the wells array (green), interconnecting plumbing routes (gray) multiple input reservoir ports (red) and output reservoir port (pink).

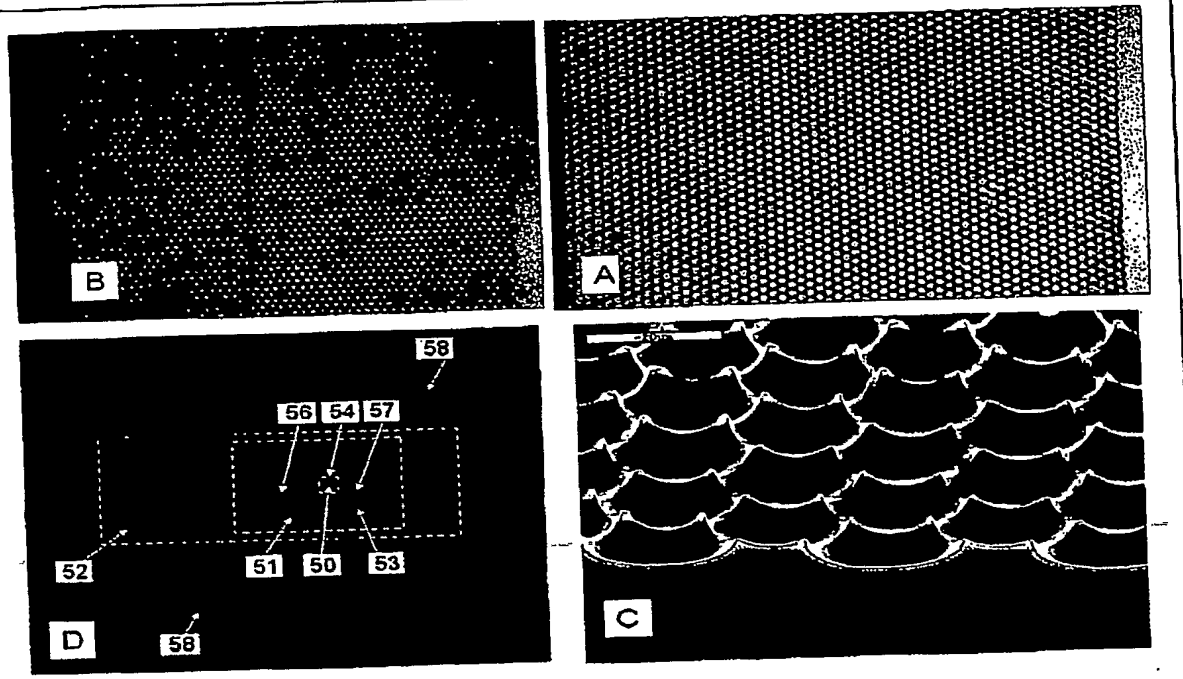

Figure 3:
(1st) A close-up of wells array (50) on P-ITICBP (made of PDMS).
(2nd) A close-up of wells array on standard glass ITICBP.
(3rd) A SEM micrograph of PDMS ITICBP (scale: 20microns).
(4th) A sample of P-ITICBP with a simple integrated fluid management system using two manual syringes. The PDMS slab (51) integrates an inner compartment (54) which harbors the wells array (50). An input (56) and output (57) internal plumbing routes accept flexible capillary tubings. The slab is placed on a standard microscope slide (52)

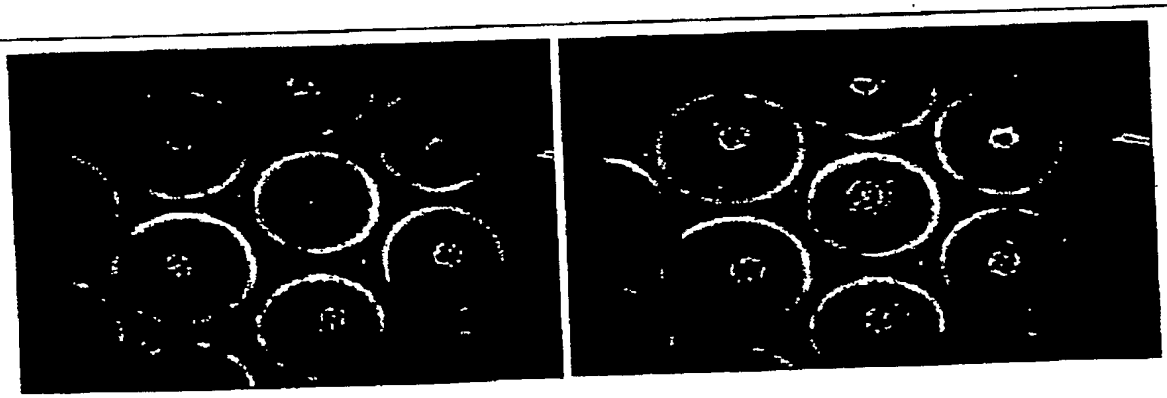

Figure 4:
A sample of cells manipulation in the P-ITICBP using laser tweezers.
6 individual lymphocites are precisely arranged in the P-ITICBP, forming a circle (right).
By using the laser tweezers again, another cell was brought from the vicinity to the central well (left).

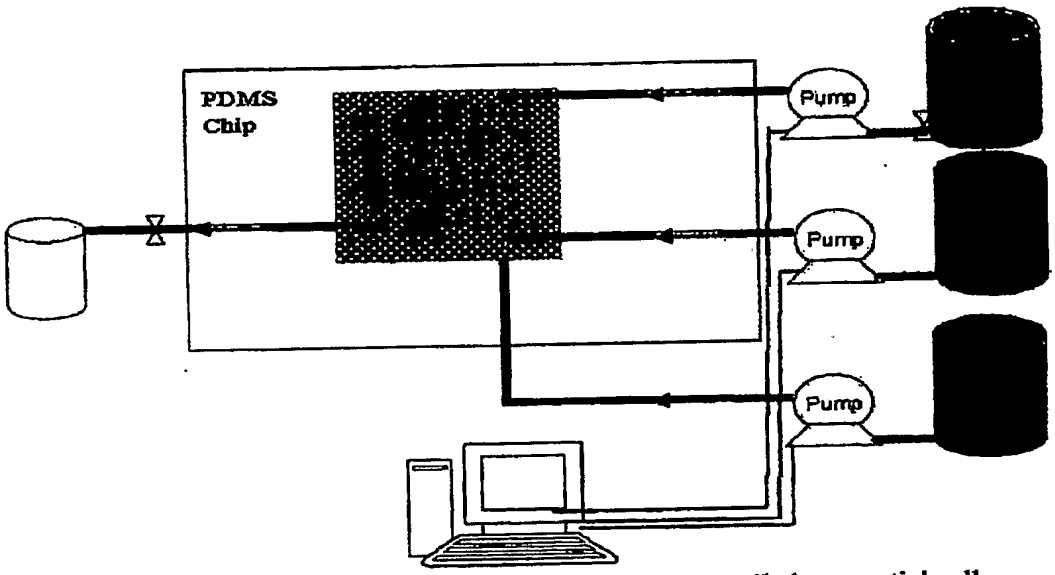

Figure 5: A demonstration of a P-ITICBP application for controlled sequential cell staining and flushing.

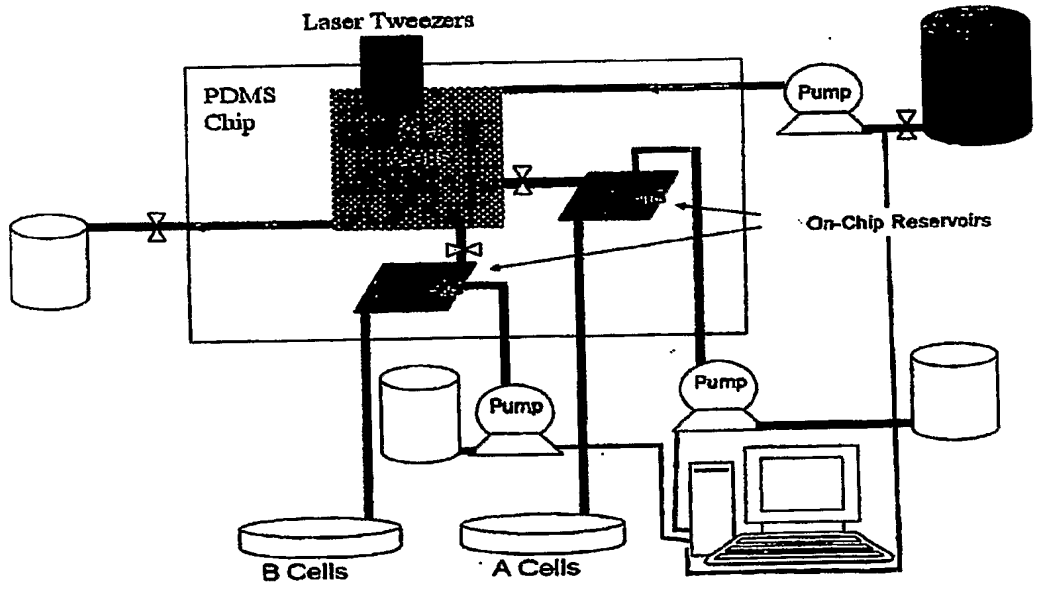

Figure 6: A demonstration of a P-ITICBP application for cell sorting utilizing computer controlled Laser Tweezers and micro-fluidics.